

## EXAMPLE 1

A sterile 1.6% solution of high G sodium alginate in 0.9% saline is obtained from Protan Labs (Pronova LV G) via Irvine Scientific. The solution has a pH of 7.02, an osmolarity of 311 mosm/kg water, and an endotoxin content less than 1.2 EU/ml as determined by Limulus Amoebocyte Lysate (LAL). 0.5 cc of this solution is injected into each of 4 stifle joints of two New Zealand White rabbits (NZW). After 2 and 4 days one rabbit is sacrificed and the stifle opened for gross evaluation, cytological evaluation, and histological evaluation. At two days the sodium alginate is grossly present in the stifle joint. The majority of the material appeared in the posterior pouch. Material is also noticed in the long digital extensor sheath. Neither cytology of the synovial fluid nor histology indicated any adverse reaction of the tissue to the sodium alginate. At 4 days post op, there is no gross indication of any material present in the stifle. Cytological evaluation indicates no adverse reaction to the material. Histology indicates no adverse reaction to the material, and shows a coating of the sodium alginate over the tissue sections analyzed (synovial membranae, articular cartilage, and ACL).

## EXAMPLE 2

A 0.25% solution is prepared by addition of 5 grams of sodium alginate (Pronova MV G, Protan) to 2 liters of 0.15M PBS. The resulting solution is filtered through a 0.45/0.22 micron filter capsule (Sartorius). The solution is then filtered through a 0.2 micron Endotoxin Affinity Membrane (EAM) (AlerCHEK, Portland, Me.) via a tangential flow process. Both the filtrate and retentate are subsequently collected and the equipment depyrogenated. This procedure is repeated for ten cycles. Following the tenth cycle, the solution (approximately 1.9 liters) is then rendered free of all low molecular weight impurities by extensive dialysis (ten cycles—pump down to 300 ml and reconstituted to 1,900 ml) with 0.5% saline via a 30K molecular weight cutoff membrane (Filtron, Norwood, Mass.) on the same ultrafiltration equipment. On the final pump down step the material is concentrated to 118 ml (4% alginate in 0.5% saline) and sterile filtered through a 0.22 micron membrane. Pyrogen concentration is determined to be 6.3 EU/ml in a 4% solution. A 4 day intra-articular injection of 0.5 cc of the purified materials is conducted in two stifle joints of a NZW. Evaluation of the tissues via gross, cytology, and histology indicated no adverse reaction to the material.

To test the adhesive prevention ability of the alginate, the fat pad from the stifle joint of the rabbit is removed and the tibia abraded. The joint is immobilized for 21 days. Two NZW rabbits serve as controls and are injected with 0.9% saline following closure. Three other animals are injected with the sodium alginate solution prepared in Example 2. All three are injected with 0.5 cc at closure (time 0). One is reinjected with 0.5 cc of the alginate solution at 7 days post op (time 0 and 7) and the third is injected at 4, 8, and 12 days post op (time 0, 4, 8, 12). All five animals are sacrificed at 21 days. Control animals show excellent adhesion formation upon gross and histological evaluation. Results of adhesions from the control subjects indicate no difference in quality or quantity of adhesion formed upon gross evaluation or histological evaluation when compared to the treated knee. Histology indicates that sodium alginate is present coating the tissues in all three test subjects.

These results indicate that a thin coating of only the alginate material is not sufficient to prevent or reduce intra-articular adhesions.

## EXAMPLE 3

A complexing solution is now utilized to cross-link the alginates of Examples 1 and 2. Two NZW rabbits are used as test subjects. Injections are made into both stifle joints of the two rabbits. One stifle receives a simultaneous injection of 0.5 cc sodium alginate (Example 1) and 0.2 cc of 2% calcium chloride from two separate syringes placed within the stifle joint. A second joint receives simultaneous injection of 0.5 cc of 0.16% sodium alginate and 0.3 cc of 2% calcium chloride. A third stifle joint receives a simultaneous injection of 0.5 cc of sodium 0.16% alginate and 0.4 cc of 2% calcium chloride, and the final joint receives 0.5 cc of 0.16% alginate and 0.5 cc of 2% calcium chloride. The purpose of this is two-fold; 1) to determine the effect of different volumes of calcium chloride on gel strength in the joint and 2) evaluate the inflammatory response to the calcium alginate gel formed in situ. The gross evaluation at two days post op indicates no inflammatory reaction to any mixture. This is confirmed by cytology and histology. There appears to be a correlation between gel strength and gel presence with increasing calcium chloride volume. The 0.5 cc of calcium chloride also seems to produce a calcium alginate gel within the stifle which almost produces an impression of the joint.

The test for adhesion preventions set forth above is performed by injecting the four solutions of Example 3 into four prepared stifle joints of NZW rabbits. After twenty days the stifle joints of the rabbits are examined. In the first rabbit, the gel is still present in the joint. It is red in color from entrapping blood at the time of surgery. There are no adhesions and the joint surfaces and surrounding synovium look fine. Similar findings are seen in the second rabbit, except that the gel remains clear. A few thin adhesions from leftover hemorrhage are seen, but these break upon light touching with a probe. Again, the joint looks healthy. The material has spread out from beyond the region of the fat pad along the sides of the condyles. The material is friable but slippery. In comparison to the experience with fat pad replacement, it appears that the stifles from these animals are healthier and have less adhesions. Tissues and the stifle joint are then collected for histology.

The solutions of the present invention may be injected into joints other than the knee, such as the elbow, shoulder and also into the spine. Drugs or other therapeutic agents such as antibiotics or anti-inflammatory agents may be included in the solution.

In addition to calcium chloride, other water soluble cations may be used as the complexing solution such as MgCl, CaSO<sub>4</sub>, MgSO<sub>4</sub>, etc. Concentrations of these complexing solutions range from about 0.5% to 2%.

While several examples of the present invention have been described, it is obvious that many changes and modifications may be made thereunto, without departing from the spirit and scope of the invention. This includes first injecting the complexing solution and thereafter injecting the alginate solution.

We claim:

1. A method for preventing adhesions in situ between tissues after surgery comprising the steps of: